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# Early Childhood Cytomegalovirus (CMV) Infection and Children's Neurocognitive Development

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**Abstract (308 words)**

**Main text (3908 words)**

**ABSTRACT**

BACKGROUND: Despite a clear association seen in congenitally infected children, the effect of postnatal cytomegalovirus (CMV) infection during early childhood on cognitive development has not yet been determined.

METHODS: CMV infection status was obtained based on serological measurements when children were 7 years old. Using population-based longitudinal data, we employed multivariate Poisson regression with a robust variance estimator to characterize the relationship between childhood CMV infection and adverse neurocognitive outcomes in children. Suboptimal neurocognitive outcomes were compared between CMV-positive and CMV-negative children using various cognitive assessments from 8 to 15 years of age. Children were evaluated on the cognitive domains of language, reading, memory, and general intelligence; with a suboptimal score being greater than 2 standard deviations lower than the mean score. Approximate Bayes factor (ABF) analysis was used to determine the level of evidence for the observed associations.

RESULTS: With adjustment for potential confounders, we observed that early childhood CMV infection was associated with suboptimal total intelligence quotient (IQ) at 8 years of age (incident rate ratio (IRR)=2.50, 95% CI 1.35-4.62, ABF=0.08); however, not with suboptimal total IQ at 15 years of age (IRR=0.97, 95% CI 0.43-2.19, ABF=1.68). Suboptimal attentional control at 8 years (IRR=1.74, 95% CI 1.13-2.68, ABF=0.18) and reading comprehension at 9 years (IRR=1.93, 95% CI 1.12-3.33, ABF=0.24) were also associated with CMV infection. Approximate Bayes factor analysis provided strong evidence for the association between CMV infection and total IQ at 8 years and only anecdotal evidence for attentional control at 8 years and reading comprehension at 9 years. All other cognitive measures assessed were not associated with CMV infection.

CONCLUSIONS: In this large-scale prospective cohort, we observed some evidence for adverse neurocognitive effects of postnatal CMV infection on general intelligence during early childhood, however not with lasting effect. If confirmed, these results could support the implementation of preventative measures to combat postnatal CMV infection.

**Key words:** cytomegalovirus infection, child neurodevelopment, ALSPAC

**Key Messages**

- **Adverse neurological and cognitive effects of congenitally acquired CMV infection have been well established; however the relationship remains unclear for postnatally acquired infection.**
- **Among a large population-based longitudinal cohort, we observed that early childhood CMV infection is associated with intellectual impairment in later childhood based on total IQ, however not with lasting effect.**
- **We did not find strong evidence that early childhood CMV infection is associated with the cognitive domains of language, attention, reading, or memory in later childhood.**
- **If the observed associations are confirmed, it could provide further support for the implementation of preventative measures to combat postnatal CMV infection, particularly within early childhood.**

## INTRODUCTION

Cytomegalovirus (CMV) is the most common congenital and perinatal infection worldwide (1). It represents a widespread viral infection, but remains largely unrecognised globally (2, 3). CMV may be acquired in utero (congenital CMV infection), during the birth process due to exposure to infected maternal secretions (sometimes referred to as perinatal CMV infection), or after birth (postnatal CMV infection) (4). Postnatally acquired CMV infections, most commonly resulting from transmission through breast milk, but also through blood transfusions or other fluid contact with infected individuals, are significantly more prevalent than congenitally acquired infections (1). However, the majority of what is known about CMV-associated health outcomes is in relation to the congenital form, whose prevalence is approximately 0.6-0.7% in developed countries, and is estimated to be much higher in developing nations (5-9). It is one of the most common causes of congenital sensorineural hearing loss and has been associated with a wide range of adverse neurodevelopmental outcomes such as vision loss,

cerebral palsy, microcephaly, as well as motor, behavioural and cognitive deficits (5, 8, 10, 11). It has been estimated that upwards of 60% of the human population is infected with CMV by adulthood, with infection occurring primarily throughout childhood and adolescence (12). Seroepidemiologic studies have shown that CMV antibody prevalence is influenced by age, geography, cultural factors, socioeconomic status, and child-rearing practices (13).

The extent of the health-related consequences associated with postnatally acquired CMV infection is far from clearly understood for the following reasons: (i) the delayed onset of adverse health sequelae makes the establishment of causal associations difficult; (ii) as postnatal infection is largely asymptomatic, it has received little research attention compared to the more severe congenital form; and (iii) while screening tests have recently become available in few countries (e.g. Canada), it is limited to the congenital form of CMV, leaving asymptomatic postnatal infection undetected. In addition, universal newborn CMV screening remains a contentious point and is currently not implemented in most countries, despite community advocacy and the recent consensus report from an international panel of experts (3). As CMV is known to be neurotropic, it is of great importance to comprehensively investigate the effects of postnatally acquired infection.

Previous studies have suggested that CMV infection during early postnatal life could impair complex cognitive skills in later childhood (14, 15). For the present investigation, our primary goal was to comprehensively investigate the association between postnatal CMV infection and a full

spectrum of adverse neurocognitive outcomes, as well as to characterize factors strongly associated with postnatal CMV infection for target surveillance. We hypothesized that children with postnatally acquired CMV infection, when compared to uninfected children, more commonly have impaired neurodevelopment and suboptimal cognitive abilities in later childhood.

## METHODS

### DATA COLLECTION AND STUDY POPULATION

Population-based data were obtained from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort (16). ALSPAC is a transgenerational prospective birth cohort study of children born in the former county of Avon in the Bristol area of the UK during 1990-92. It is one of the most detailed studies of its kind in the world, with an immense variety of health information arising from questionnaire and clinical data collection over two decades. It includes genetic, epigenetic, biological, psychological, social and other environmental determinants in relation to a similarly diverse range of health, behavioural and developmental outcomes. The source data and characteristics of the cohort have been described in further detail elsewhere (17, 18). Please note that the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool (18).

A flow diagram of participant recruitment is presented in Figure 1. Among the 15 589 children enrolled in ALSPAC, 5007 had serological CMV

measurement at 7 years and were included in the study population. We further excluded children with severe medically-diagnosed neurological conditions (e.g. epilepsy, cerebral palsy) and those who were not enrolled in school at either 6 years or 9 years of age, which resulted in 4988 children in the analysis. These criteria were considered as they may have had a large and undue influence on children's cognitive outcomes. The population that had CMV serological data are comparable with the overall ALSPAC population in terms of children's gender distribution and method of delivery, but different in other aspects (Supplementary Table 1), with higher education levels and longer breastfeeding duration among other factors.

(Figure 1 here)

**EARLY CHILDHOOD CMV INFECTION**

The earliest CMV serological data available from ALSPAC with sufficient sample size for statistical analysis was at 7 years of age. Infection was measured with three associated variables of CMV IgG antibody titers: a raw optical density, a ratio-to-standard, and a normalised z-score of that ratio-to-standard. Methods used for IgG antibody measurement were based on standard enzyme-linked immunosorbent assay (ELISA) techniques which were performed previously (19, 20). CMV IgG antibody titers showed a bimodal frequency distribution, thus we used finite mixture modelling methods that were previously described to determine a cut-off threshold value for CMV positivity (21). For purposes of this study, the CMV ratio-to-standard optical density measurement was used to characterize

infection as it had the most distinct bimodal distribution for threshold determination.

**NEUROCOGNITIVE OUTCOMES**

Neurocognitive impairment, the primary endpoint we aimed to measure, is clinically defined as a reduction in cognitive functioning in one or more cognitive domains, including memory, attention, learning, language, perception, and social cognition (22). A variety of neurocognitive measures were available from the ALSPAC cohort, and were chosen based on demonstrated ability to characterise different dimensions of cognitive capacity. We chose to evaluate neurodevelopment based on the cognitive domains of intelligence, language, reading, memory, and attention using the Wechsler Intelligence Scale for Children (WISC), the Wechsler Objective Language Dimensions (WOLD), the Neale Analysis of Reading Ability (NARA), the Counting Span Task, and the Test of Everyday Attention for Children (TEA-Ch), respectively. All cognitive assessments were administered by experienced and trained psychologists (16).

The WISC-III was used to characterise children's intellectual ability at 8 years as it is the most widely used individual intelligence test worldwide and is well documented in its ability to assess different aspects of intelligence for children between the ages of 6 to 16 (23-25). A subset of the ALSPAC cohort (n=7488) attended the focus clinic at 8 years and completed the intelligence assessment using a short form of the WISC-III test. The assessment consisted of subtests designed specifically for measurement of verbal intelligence quotient (IQ), performance IQ, and total IQ, which were calculated from each of the

subtests' total age-scaled scores. An additional intelligence measurement was taken at 15 years (n=5,509) using the Weschler Abbreviated Scale for Intelligence (WASI). This assessment consisted of a measurement for vocabulary, matrix reasoning, and total IQ.

The language dimensions of listening comprehension and oral expression were measured using WOLD subtests at the focus clinic at 8 years of age (n=7488) (26). WOLD consists of individually administered tests designed for children aged 6 to 16 years.

TEA-Ch was used to measure different aspects of attention at 8 years and 11 years, including: selective attention, the ability to divide attention between two tasks, and attentional control (27). TEA-Ch has been documented as a valuable tool in assessing specific attentional operations and is specifically useful in characterising attentional disorders in children between the ages of 6 and 16 (28). The testing was performed at ALSPAC focus clinics at 8 years of age (n=7488) and 11 years of age (n=7159).

Reading accuracy and comprehension were measured at 9 years using the NARA-II, which is a reading assessment based on a series of short narratives suitable for children between the ages of 6 and 12 (29). Assessment was administered at the ALSPAC focus clinic at 9 years of age (n=7725). Words per minute, accuracy and comprehension standardised scores were calculated. Additionally, a classification of developmental dyslexia was provided by ALSPAC using a clinical definition of the disorder.

Lastly, working memory was tested using the Counting Span Task at 10 years of age (n=7563). Working memory was an important cognitive component to assess as it has been implicated as a crucial ability in reading and arithmetic. Working memory tasks require the simultaneous processing and storage of information (30). The Counting Span Task is a widely accepted measure of working memory capacity with documented reliability and validity (31).

For each of the composite scores measuring different aspects of cognitive functioning described above, threshold values were chosen in order to divide subjects into categories of 'suboptimal' and 'normal' neurocognition. A suboptimal threshold value of more than 2 standard deviations (SDs) below the mean score was used for each measurement type (in cases where a high score indicated suboptimal, a threshold value of more than 2 SDs above the median score was used).

Ethical approval for the ALSPAC study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time. In addition, this project was approved by the Research Ethics Board at Mount Sinai Hospital.

**CONFOUNDERS**

Potential confounders known to influence cognitive development were selected a priori for inclusion in the regression model, including the child's sex (32), ethnicity (33), gestational age (34), duration of breastfeeding (35), and the quality of home environment (36), as well as the mother's use of potential harmful substances (e.g. tobacco, alcohol, drugs) during pregnancy (37), education levels (as a measure of socioeconomic status) (38), and psychological condition (39). We used an adaptation of the Home Observation Measurement of the Environment (HOME) scoring measure to evaluate aspects of the quantity and quality of social, emotional, and cognitive support made available to the child in their home environment (40). For greater accuracy of this score, we took the average of two measurements which were collected when children were 6 months and 18 months of age. The mother's psychological condition after giving birth was assessed when the child was 8 months old using the Edinburgh Postnatal Depression Scale (EPDS) (41). Lastly, mothers' use of illicit drugs and cigarettes during pregnancy were considered as binary (ever/never), and levels of alcohol use during pregnancy were considered (none/moderate/heavy) by combining data from multiple collection time points and taking the average.

**STATISTICAL ANALYSIS**

For purposes of target surveillance and prevention, we applied a multivariate Poisson regression with a robust variance estimator to determine factors strongly associated with CMV postnatal infection (measured at 7 years). Exposures investigated included: method of delivery, pregnancy size (i.e., singleton vs twin), infant school

attendance, biological sex, gestational age, birthweight, HOME score, breastfeeding duration, mother's education, ethnicity, age at delivery, EPDS, vitamin supplementation during pregnancy, and use of harmful substances (tobacco, alcohol, drugs) during pregnancy. In order to assess how factors influence performance on neurocognitive measures independently of CMV infection and to evaluate the potential for strong confounding, we also conducted a secondary analysis among CMV-negative children where we examined the associations between potential confounders and neurocognitive outcomes of interest.

We employed multivariate Poisson regression with a robust variance estimator to estimate the incidence rate ratio (IRR), and 95% confidence interval (CI) to evaluate the association between CMV seropositivity at 7 years of age and suboptimal cognitive outcomes. The biological sex of the child was then assessed as a potential effect modifier by stratified analysis.

Imputation was used to limit missingness of the covariates in the regression models. When missing, mother's education, breastfeeding duration and average HOME score were imputed to the median value of each variable for those with CMV serological data (i.e., the analysis subpopulation). Statistical analysis was performed using Stata statistical and data analysis software version 15.0 (42).

**APPROXIMATE BAYES FACTOR ANALYSIS**

In order to mitigate the potential issue of multiple comparisons, we estimated the approximate Bayes factor (ABF) based on methods previously

described to assess the noteworthiness of observed associations (43-45). An ABF can be interpreted as a measure of the strength of evidence in favour of the alternative hypothesis compared to the null and does not depend on the number of comparisons. An ABF of less than 0.1 or greater than 10 is considered strong evidence for the alternative hypothesis; 0.1-0.33 or 3-10 is moderate evidence; 0.33-1 or 1-3 is anecdotal evidence; and 1 is no evidence (46).



## RESULTS

### *Study Population and Factors Associated with CMV Infection*

Demographic data on the study population is summarized in Table 1. There were 4988 participants that had serological CMV data at 7 years of age and met inclusion criteria for the analysis. Overall, 51.8% were boys, 98.3% were from mothers with European ancestry, and mean gestational age was 39.4 weeks. Of these participants, 864 (17.3%) were classified as CMV 'positive' (CMV+; infected) and 4124 (82.7%) were classified as CMV 'negative' (CMV-; uninfected) based on the threshold value computed using IgG antibody titers. Children in the CMV+ and CMV- groups were comparable on most child and maternal parameters. Table 1 also summarizes the mutually adjusted factors associated with CMV infection. As reported in previous studies (47), longer breastfeeding duration was shown to be associated with CMV infection. Mothers of non-European ancestry were found to have a positive association with children's CMV infection when compared to mothers with European ancestry (IRR=2.34, 95% CI 1.52-3.59).

Furthermore, twin pregnancy was found to have an inverse association with CMV infection (IRR=0.40, 95% CI 0.17-0.95).

(Table 1 here)

***Factors Associated with Neurocognitive Outcomes***

Table 2 summarizes the associations between potential confounding factors and the primary outcome of interest, WISC total IQ at 8 years, in the absence of CMV infection. There was strong evidence for associations between HOME score and suboptimal WISC total IQ at 8 years (IRR=0.64, 95% CI 0.53-0.77, ABF<0.01). The associations between potential confounders and other cognitive outcomes are summarized in Supplementary Tables 2a-d. In brief, we observed associations between breastfeeding duration (6+ months), alcohol use during pregnancy (>7 units per week) and TEA-Ch attentional control at 8 years (IRR=0.40, 95% CI 0.21-0.75, ABF=0.09; IRR=2.34, 95% CI 1.31-4.18, ABF=0.09, respectively), and child's sex with NARA reading comprehension at 9 years (IRR=0.31, 95% CI 0.15-0.63, ABF=0.05).

(Table 2 here)

***Effects of CMV Infection on Neurocognition***

Table 3 summarizes the results of the association of CMV with children's neurodevelopment. After adjustment for potential confounders, CMV infection was associated with suboptimal WISC total IQ at 8 years (IRR=2.50, 95% CI 1.35-4.62, ABF=0.08) but not with WASI total IQ at 15 years (IRR=0.97, 95% CI 0.43-2.19, ABF=1.68). CMV infection was also associated with suboptimal attentional control using the TEA-Ch at 8

years (IRR=1.74, 95% CI=1.13-2.68, ABF=0.18), but not at 11 years (IRR=1.07, 95% CI 0.65-1.77, ABF=2.33). Based on the NARA at 9 years, CMV infection indicated a higher risk for suboptimal reading comprehension (IRR=1.93, 95% CI 1.12-3.33, ABF=0.24). All other measures of intelligence, attention and reading were not associated with CMV infection. Similarly, measures of suboptimal language dimensions and working memory were not found to be associated with CMV infection. ABF analysis suggested that there was strong evidence for the association between CMV infection and lower WISC total IQ at 8 years, but only moderate evidence for suboptimal TEA-Ch attentional control at 8 years and NARA reading comprehension at 9 years.

(Table 3 here)

We further assessed whether the associations differed by the sex of the child. Results of the sex-stratified analysis and we observed no differences between boys and girls on any cognitive assessments (Supplementary Table 3).



**DISCUSSION**

To our knowledge, this is the largest and longest prospective longitudinal analysis investigating neurocognitive outcomes in children infected postnatally with CMV. Our analysis supported previous associations reported regarding longer breastfeeding duration and ethnicity being major influential exposures for postnatal CMV infection (48). Our results provide some evidence for adverse neurocognitive

effects of postnatal CMV infection on total IQ during early childhood, but this effect may be short-lived as subsequent measurement of total IQ in later adolescence yielded no association. Overall, postnatal CMV infection did not appear to influence cognitive performance on the majority of other measures that were assessed.

It is widely recognized that symptomatic congenital CMV infection has widespread neurological and developmental consequences (6), however few have studied either asymptomatic congenital CMV infection or postnatal infection. In particular, previous studies focusing on asymptomatic congenital CMV and neurodevelopment have found inconsistent associations (49-53), and postnatal CMV infection and neurodevelopment has only been studied in higher-risk subpopulations, such as preterm infants to date (14, 54-56). Overall, results have been inconclusive and highlight the gap in current knowledge related to CMV infection and cognitive impairment.

There are various proposed mechanisms as to how CMV infection may impair neurological and cognitive abilities. A recent study of postnatal CMV infection in preterm infants using sophisticated MRI analyses found that subtle neural alterations (e.g., regional differences in functional activation or disturbances within the neural networks) may explain the neurological sequelae in infected individuals (14, 47). It has also been hypothesized that the observed association between CMV and intelligence could be mediated by leukocytes. Higher CMV IgG titres have been associated with lower leukocyte telomerase activity and shorter leukocyte telomere length in healthy adults, while longer leukocyte telomeres were

associated with superior cognitive performance in a large meta-analysis (57-59). Finally, CMV infection has also been shown to modulate inflammatory and anti-inflammatory circulating biomarkers (e.g., cytokines, receptor antagonists, immune cells, and metabolic markers) and in turn, these neuroimmune factors may increase neurodegeneration and affect cognitive performance (55).

It has been previously concluded that sex modulates neuroimmune factors, cognitive performance, and the relationship between the two domains (50, 51, 55). Therefore, we sought to understand how biological sex impacted the associations between CMV infection and neurocognitive measures. The sex-stratified analysis suggested there is no differential effect of postnatal CMV infection on cognition between boys and girls.

It should be noted that cognitive domains are not independent of one another as domains of cognitive functioning are hierarchical in nature, ranging from basic sensory and perceptual processes to higher executive functioning and cognitive control. Therefore, scores on different measures may be inter-related based on the same underlying mechanisms of neurocognitive development. For example, language deficits may be associated with deficits of executive functioning and processing speed (60). Further, interrelationships between reading and intelligence are widely recognized, especially in children (61, 62). For example, we observed correlation between WISC at 8 years of age and NARA reading comprehension at 9 years of age (Pearson correlation= 0.64, $p<0.05$), which is also reflected in their associations with CMV infection. The specific tests of different domains may provide information on each

domain, while the global executive functioning is the general representation that involved each domain (60).

Although we observed an association between CMV infection and neurocognitive impairment using WISC total IQ at 8 years of age, we did not observe the same impairment in WASI total IQ at 15 years of age. There are several possible reasons that could explain these differences over time: (i) children scoring sub-optimally at the 8-year measurement were less likely to continue to participate in measurement at the later time point than children who scored within normal range (44% vs. 63% retention, respectively); (ii) cognitive impairment due to CMV infection may not be long-lasting (53, 63). In other words, CMV infection may cause a deficit in cognitive domains during early childhood; however it is possible that infected individuals may compensate cognitively by adolescence; and/or, (iii) CMV seropositivity status at 7 years captures all children who contracted CMV in utero up to age 7. Given the extended lag time needed between CMV infection and adverse neurodevelopment sequelae, the cognitive impairment observed at 8 years is likely due to CMV infection during the toddler time period (critical for brain development), whereas the effect of later CMV infection is likely to be reflected in cognitive measurements at 11 or 15 years old. Therefore, the alternative hypothesis of this observation is that only postnatal infection within infancy or the toddler period, when the brain tissue is under active development, would have an effect on cognitive impairment (as manifested at 8 years), and possibly not CMV infection acquired later on at school age. However, given the timing of the CMV measurement, we are not able to tease out the susceptibility time period in this analysis,

which is a limitation. One related limitation is that we were also not able to distinguish congenital CMV infection from early childhood CMV infection. However, congenital CMV infection is rare at merely 0.6-0.7%, compared to up to 20% prevalence of CMV infection in childhood. Thus, while we do not expect congenital infection to be driving the association we observed, we cannot preclude this possibility. It is important for our results to be confirmed in a future study where differentiation between congenital and postnatal CMV is possible. For future investigations, it would be best to have multiple earlier serological measurements of CMV antibodies within the first years of life in order to identify the timing of infection and the susceptible time window related to potential adverse sequelae later on.

Another limitation of using data from this specific UK-based cohort was the ethnic homogeneity of the study population. The total proportion of mothers with European ancestry recruited eclipsed that of non-Caucasian races (~97% Caucasian vs ~3% all other ethnicities combined), making a detailed analysis on ethnic subgroups impossible. Lastly, it is possible that children's overall health status is related to viral antibody levels, and it may influence a child's performance on cognitive tests. We did not have data on the overall health status of children and were therefore unable to characterise this relationship or account for it in our modelling. However, it is likely that overall health status would mediate, rather than confound, the relationship between CMV infection and suboptimal neurocognitive outcomes. Therefore it is unlikely to result in a biased association when unaccounted for.

The key strengths of this study are the size of the study population, the length of follow-up and the extent of cognitive assessments that were conducted prospectively. Previous studies examining postnatal CMV infection have primarily focused on much narrower study populations arising from single-centre recruitment. This investigation represents the largest analysis conducted for postnatal CMV infection and children's cognitive outcomes for multiple constructs at multiple time points.

In conclusion, based on a longitudinal study assessing neurocognitive sequelae of children infected postnatally with CMV, we observed a potential increased risk of suboptimal general intelligence associated with infection, although the effect may not be long-lasting. However, the majority of our findings support the null hypothesis that postnatal CMV infection is not associated with adverse neurocognition in later childhood. Nonetheless, our study provides a foundation and motive for further research into the largely understudied relationship between postnatal CMV infection and adverse neurocognitive outcomes, with specific focus on timing of susceptibility. In future investigations, measurements of IgG avidity as well as other antibody types (e.g., IgM) in addition to IgG could help to differentiate active, reactivated, and past CMV infections and shed further insights on CMV sequelae related to the susceptible time window. As CMV is the most wide-ranging and prevalent perinatal infection, and its health consequences are largely unknown, knowledge of its role in neurocognitive development could be beneficial to population health. Knowledge of children's heightened risk for adverse cognitive outcomes associated with postnatal CMV infection

is important when evaluating whether early interventions to mitigate long-term effects of CMV infection are warranted.

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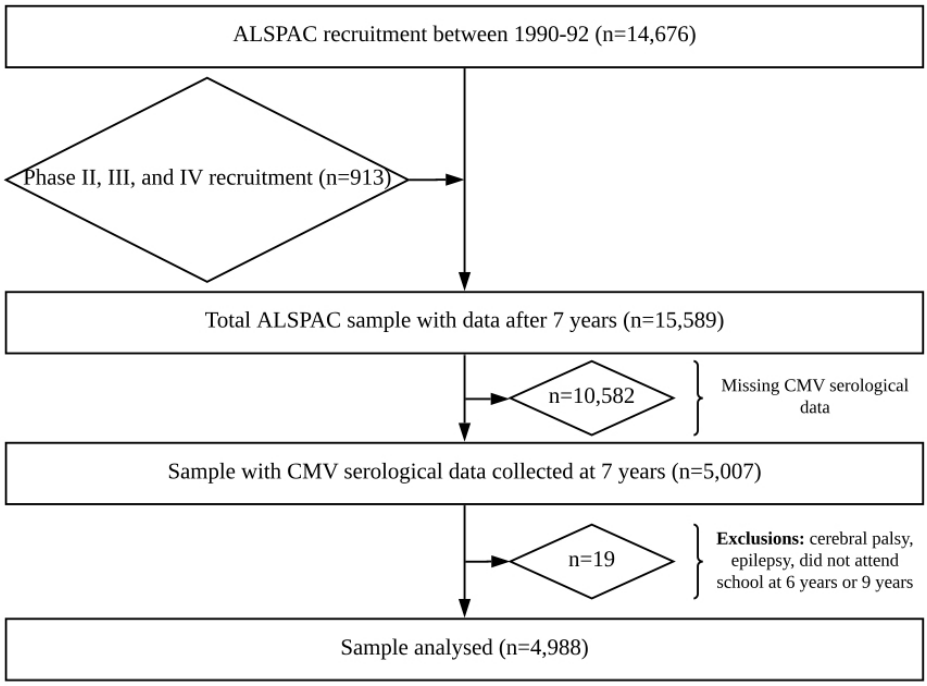


Figure 1. Flow diagram depicting study participant recruitment and exclusions

155x117mm (150 x 150 DPI)

**Table 1. Baseline Characteristics of the overall study sample by CMV infection status**

| | Overall Study Sample (n=4988)[a] | | CMV Positive (n=864) | | CMV Negative (n=4124) | | Incidence-Rate Ratio of CMV infection* | | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| | n | (%) | n | (%) | n | (%) | IRR | (95% CI) | |
| **Child Characteristics** | | | | | | | | | |
| Sex | | | | | | | | | |
| Male | 2582 | (51.8) | 447 | (51.9) | 2135 | (51.8) | 1.00 | (Reference) | - |
| Female | 2401 | (48.2) | 415 | (48.1) | 1986 | (48.2) | 0.93 | (0.78,1.12) | 0.44 |
| Gestational age, weeks, mean | 39.4 | (SD=1.8) | 39.4 | (SD=1.8) | 39.5 | (SD=1.8) | 0.97 | (0.91,1.03) | 0.34 |
| Birthweight, grams, mean | 3436.1 | (SD=545.2) | 3429.1 | (SD=542.9) | 3437.6 | (SD=545.8) | 1.00 | (1.00,1.00) | 0.57 |
| Pregnancy size | | | | | | | | | |
| Singleton | 4866 | (97.7) | 848 | (98.4) | 4018 | (97.5) | 1.00 | (Reference) | - |
| Twin | 117 | (2.3) | 14 | (1.6) | 103 | (2.5) | 0.40 | (0.17,0.95) | 0.04 |
| Breastfeeding duration | | | | | | | | | |
| Never | 845 | (19.7) | 87 | (11.7) | 758 | (21.4) | 1.00 | (Reference) | - |
| <3 months | 934 | (21.8) | 120 | (16.2) | 814 | (22.9) | 1.15 | (0.81,1.63) | 0.45 |
| 3-5 months | 777 | (18.1) | 146 | (19.7) | 631 | (17.8) | 1.61 | (1.14,2.27) | <0.01 |
| 6+ months | 1734 | (40.4) | 388 | (52.4) | 1346 | (37.9) | 1.79 | (1.30,2.46) | <0.01 |
| Attended infant school/preschool[b] | 3851 | (85.1) | 661 | (84.9) | 3190 | (85.2) | 1.04 | (0.75,1.45) | 0.82 |
| HOME score, mean[c] | 9.2 | (SD=1.7) | 9.4 | (SD=1.7) | 9.2 | (SD=1.6) | 1.05 | (0.99,1.12) | 0.11 |
| **Maternal Characteristics** | | | | | | | | | |
| Maternal age at delivery, years, mean | 29.2 | (SD=4.6) | 29.6 | (SD=4.7) | 29.1 | (SD=4.6) | 0.99 | (0.97,1.01) | 0.28 |
| Method of delivery | | | | | | | | | |
| Vaginal, spontaneous | 1688 | (61.1) | 312 | (63.3) | 1376 | (60.7) | 1.00 | (Reference) | - |
| Vaginal, assisted | 551 | (20.0) | 96 | (19.5) | 455 | (20.1) | 0.94 | (0.75,1.18) | 0.60 |
| Caesarean | 522 | (18.9) | 85 | (17.2) | 437 | (19.3) | 0.85 | (0.66,1.10) | 0.22 |
| Ethnicity | | | | | | | | | |
| European | 4433 | (98.3) | 740 | (96.4) | 3693 | (98.7) | 1.00 | (Reference) | - |
| Other ethnic group | 75 | (1.7) | 28 | (3.6) | 47 | (1.3) | 2.34 | (1.52,3.59) | <0.01 |
| Substance use during pregnancy[d] | | | | | | | | | |
| Cigarette use | 928 | (19.8) | 143 | (17.9) | 785 | (20.3) | 1.06 | (0.83,1.36) | 0.64 |
| Alcohol use | | | | | | | | | |
| <1 unit per week | 2599 | (55.6) | 426 | (53.3) | 2173 | (56.1) | 1.00 | (Reference) | - |
| 1-6 units per week | 1534 | (32.8) | 271 | (33.9) | 1263 | (32.6) | 1.14 | (0.94,1.39) | 0.18 |
| ≥7 units per week | 539 | (11.5) | 102 | (12.8) | 437 | (11.3) | 1.19 | (0.91,1.56) | 0.21 |
| Illicit drug use | 124 | (2.7) | 31 | (3.9) | 93 | (2.4) | 1.41 | (0.88,2.25) | 0.15 |
| Vitamin supplementation during pregnancy[e] | 983 | (21.2) | 184 | (23.2) | 799 | (20.8) | 0.97 | (0.78,1.21) | 0.80 |
| Mother's Education Level[f] | | | | | | | | | |
| None/CSE | 577 | (12.8) | 92 | (11.9) | 485 | (12.9) | 1.00 | (Reference) | - |
| Vocational | 382 | (8.5) | 51 | (6.6) | 331 | (8.8) | 0.78 | (0.49,1.23) | 0.28 |
| O-Level | 1567 | (34.7) | 234 | (30.2) | 1333 | (35.6) | 0.84 | (0.60,1.18) | 0.32 |
| A-Level | 1224 | (27.1) | 220 | (28.4) | 1004 | (26.8) | 0.93 | (0.66,1.31) | 0.68 |
| Degree | 770 | (17.0) | 177 | (22.9) | 593 | (15.8) | 1.11 | (0.76,1.61) | 0.59 |
| Mother's EPDS, mean[g] | 5.2 | (SD=4.5) | 5.2 | (SD=4.5) | 5.2 | (SD=4.5) | 1.00 | (0.98,1.02) | 0.66 |

*All factors were investigated for their association with CMV infection status at 7 years in a single modified Poisson model, therefore mutually adjusting for all other factors under investigation.

[a]The number of observations included in the study sample was dependent on the availability of serological CMV data.

[b]Infant school/preschool attendance was assessed as either full-time or part-time attendance when the child was 65 months (5 years, 5 months) of age.

[c]Home Observation for Measurement of the Environment (HOME) score was measured when the child was 6 months and 18 months of age, and is a score of 0 to 12 based on the quality of the home environment for child development including cognitive stimulation and emotional support measures, with a higher score indicating greater environment quality.

[d]Users of harmful substances during pregnancy included those who reported any use of cigarettes/tobacco, alcohol, or illicit drugs at any point during pregnancy. Illicit drugs included cannabis, amphetamine, barbiturate, crack, cocaine, heroin, methadone, and ecstasy.

[e]Mother's vitamin supplementation during pregnancy was assessed at both 18 weeks gestation and 32 weeks gestation.

[f]Mother's education level at 32 weeks gestation was obtained based on a 5-point UK education scale, including: none/CSE level, vocational level, O-level, A-level, and University Degree level.

[g]Mother's Edinburgh Post-natal Depression Score was assessed when the child was 8 months of age on a scale of 0 to 29, with a higher score indicating more symptoms of depression.

**Table 2. Associations between confounders and suboptimal total IQ (WISC 8 years) (among CMV-negative children)**

| Potential Confounders | Incidence-Rate Ratio of suboptimal neurocognitive outcome | | *P* value |
|---|---|---|---|
| | IRR | (95% CI) | |
| **Child Characteristics** | | | |
| Sex | | | |
| Male | 1.00 | (Reference) | - |
| Female | 0.50 | (0.23,1.08) | 0.08 |
| Gestational age, weeks, mean | 1.10 | (0.86,1.41) | 0.46 |
| Breastfeeding duration | | | |
| Never | 1.00 | (Reference) | - |
| <3 months | 0.30 | (0.08,1.12) | 0.07 |
| 3-5 months | 1.03 | (0.41,2.55) | 0.96 |
| 6+ months | 0.53 | (0.18,1.56) | 0.25 |
| HOME score, mean | 0.64 | (0.53,0.77) | <0.01 |
| **Maternal Characteristics** | | | |
| Substance use during pregnancy | | | |
| Cigarette use | 1.44 | (0.65,3.16) | 0.37 |
| Alcohol use | | | |
| <1 unit per week | 1.00 | (Reference) | - |
| 1-6 units per week | 0.45 | (0.19,1.10) | 0.08 |
| ≥7 units per week | 0.70 | (0.20,2.42) | 0.57 |
| Mother's Education Level | | | |
| None/CSE | 1.00 | (Reference) | - |
| Vocational | 1.89 | (0.66,5.41) | 0.24 |
| O-Level | 0.68 | (0.24,1.92) | 0.47 |
| A-Level | 0.30 | (0.07,1.39) | 0.13 |
| Degree | 0.17 | (0.02,1.41) | 0.10 |
| Mother's EPDS, mean | 0.96 | (0.89,1.05) | 0.38 |

**Table 3. Associations between CMV infection status and suboptimal neurocognitive outcomes (with adjustment for confounders)**

| | CMV Positive (infected) (n=864) | | CMV Negative (control) (n=4124) | | Incidence-Rate Ratio of suboptimal neurocognitive outcome[a] | | *P* value | Approx. Bayes factor |
|---|---|---|---|---|---|---|---|---|
| **Suboptimal Neurocognitive Outcome*** | n/N[b] | (%) | n/N[b] | (%) | IRR | (95% CI) | | |
| Wechsler Intelligence Scale for Children (WISC), 8 years | | | | | | | | |
| Verbal IQ | 9/718 | (1.3) | 54/3436 | (1.6) | 1.38 | (0.70,2.73) | 0.36 | 1.38 |
| Performance IQ | 11/717 | (1.5) | 44/3431 | (1.3) | 1.77 | (0.92,3.39) | 0.09 | 0.65 |
| Total IQ | 14/715 | (2.0) | 41/3423 | (1.2) | 2.50 | (1.35,4.62) | <0.01 | 0.08 |
| Verbal Comprehension Index | 9/713 | (1.3) | 56/3407 | (1.6) | 1.10 | (0.53,2.29) | 0.80 | 1.76 |
| Perceptual Organization Index | 16/682 | (2.3) | 60/3226 | (1.9) | 1.66 | (0.93,2.98) | 0.09 | 0.69 |
| Freedom from Distractibility Index | 9/699 | (1.3) | 66/3342 | (2.0) | 0.85 | (0.41,1.76) | 0.66 | 1.69 |
| Wechsler Objective Language Dimensions (WOLD), 8 years | | | | | | | | |
| Comprehension Score | 15/715 | (2.1) | 75/3448 | (2.2) | 0.94 | (0.50,1.78) | 0.86 | 1.96 |
| Expression Score | 19/710 | (2.7) | 95/3441 | (2.8) | 1.04 | (0.61,1.76) | 0.89 | 2.30 |
| Expression Score (description, direction, sequencing task) | 5/463 | (1.1) | 26/2293 | (1.1) | 1.29 | (0.49,3.37) | 0.61 | 1.41 |
| Test of Everyday Attention for Children (TEA-Ch), 8 years | | | | | | | | |
| Selective Attention Score | 32/694 | (4.6) | 105/3349 | (3.1) | 1.43 | (0.95,2.15) | 0.09 | 0.78 |
| Dividing Attention Score | 18/531 | (3.4) | 76/2603 | (2.9) | 1.23 | (0.71,2.14) | 0.45 | 1.79 |
| Attentional Control (same world) | 27/699 | (3.9) | 88/3370 | (2.6) | 1.74 | (1.13,2.68) | 0.01 | 0.18 |
| Attentional Control (opposite world) | 15/697 | (2.2) | 48/3368 | (1.4) | 1.65 | (0.90,3.02) | 0.11 | 0.75 |
| Neale Analysis of Reading Ability (NARA), 9 years | | | | | | | | |
| Words per Minute Score | 15/653 | (2.3) | 88/3094 | (2.8) | 1.05 | (0.59,1.87) | 0.88 | 2.13 |
| Accuracy Score | 20/654 | (3.1) | 87/3102 | (2.8) | 1.28 | (0.75,2.16) | 0.36 | 1.64 |
| Comprehension Score | 17/654 | (2.6) | 66/3102 | (2.1) | 1.93 | (1.12,3.33) | 0.02 | 0.24 |
| Developmental dyslexia[c] | 22/734 | (3.0) | 72/3456 | (2.1) | 1.55 | (0.92,2.63) | 0.10 | 0.79 |
| Counting Span Task (Working Memory), 10 years | | | | | | | | |
| Working Memory Global Score[d] | 39/677 | (5.8) | 151/3120 | (4.8) | 1.35 | (0.93,1.97) | 0.12 | 1.04 |
| Working Memory Span Score | 8/677 | (1.2) | 47/3120 | (1.5) | 0.84 | (0.40,1.77) | 0.65 | 1.66 |
| Test of Everyday Attention for Children (TEA-Ch), 11 years | | | | | | | | |
| Selective Attention Score | 17/661 | (2.6) | 92/3156 | (2.9) | 1.00 | (0.56,1.77) | 0.99 | 2.17 |
| Dividing Attention Score | 6/650 | (0.9) | 32/3113 | (1.0) | 1.18 | (0.50,2.77) | 0.71 | 1.56 |
| Attentional Control (same world) | 23/646 | (3.6) | 100/3032 | (3.3) | 1.07 | (0.65,1.77) | 0.79 | 2.33 |
| Attentional Control (opposite world) | 26/645 | (4.0) | 91/3031 | (3.0) | 1.55 | (0.98,2.43) | 0.06 | 0.55 |
| Wechsler Abbreviated Scale of Intelligence (WASI), 15 years | | | | | | | | |
| Vocabulary Score | 14/523 | (2.7) | 63/2438 | (2.6) | 1.16 | (0.60,2.26) | 0.66 | 1.80 |
| Matrix Reasoning Score | 19/523 | (3.6) | 86/2436 | (3.5) | 1.21 | (0.73,2.02) | 0.45 | 1.90 |
| Total IQ | 7/523 | (1.3) | 44/2435 | (1.8) | 0.97 | (0.43,2.19) | 0.94 | 1.68 |

*Each suboptimal neurocognitive outcome was analysed in a separate multivariate modified Poisson regression model, adjusting for the child's sex, ethnicity, gestational age, duration of breastfeeding, HOME score, mother's education, mother's postnatal psychological condition (using EPDS), and mother's use of tobacco, alcohol, and illicit drugs during pregnancy.

[a]Incidence-rate ratio of the suboptimal neurocognitive outcome for those who are CMV positive, compared to those who are CMV negative (i.e., CMV negative is the reference group).

[b]Proportion of observations with suboptimal neurocognitive outcome, defined by 2 SDs below (or above, if a high score indicates the adverse outcome) the mean score for the entire cohort.

[c]The definition of developmental dyslexia was devised by Julie Williams and Alan Emond and is based on the accuracy component of the NARA II. The equivalent reading age based on the accuracy score is calculated from the NARA II manual and the difference between this and the actual age is calculated. Where the child's reading age is greater than or equal to 30 months (2.5 years) behind the actual age AND the child's WISC Total IQ is greater than or equal to 85 the child is considered developmentally dyslexic.

[d]Suboptimal working memory global score was defined by ≤5th percentile, as 2 SDs below the mean yielded a threshold that was too stringent (i.e., there were <5 observations in any cell for comparison in the overall sample).